

PATENT COOPERATION TREATY

PCT

REG'D 07 DEC 2004

INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference HTR 00128/WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/08446	International filing date (day/month/year) 30.07.2003	Priority date (day/month/year) 30.07.2002
International Patent Classification (IPC) or both national classification and IPC G01N15/14		
Applicant STEINBEIS-TRANSFERZENTRUM		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the opinion
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 26.02.2004	Date of completion of this report 06.12.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bravin, M Telephone No. +49 89 2399-2417



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP 03/08446

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-12 as originally filed

Claims, Numbers

1-14 received on 30.07.2004 with letter of 28.07.2004

Drawings, Sheets

1/10-10/10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP 03/08446

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	
	No: Claims	1-14
Inventive step (IS)	Yes: Claims	
	No: Claims	1-14
Industrial applicability (IA)	Yes: Claims	1-14
	No: Claims	

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP 03/08446

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.

Reference is made to the following documents:

- D1: MONTEIRO-LEAL ET AL: "Gold finder: a computer method for fast automatic double gold labeling detection, counting, and color overlay in electron microscopic images" JOURNAL OF STRUCTURAL BIOLOGY, vol. 141, - 7 February 2003 (2003-02-07) pages 228-239, XP002274390
- D2: WO 01/04828 A (ELLIS BOB ;MCLAREN GINA (US); CHROMAVISION MED SYS INC (US)) 18 January 2001 (2001-01-18)

2.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-14 is not new in the sense of Article 33(2) PCT.

2.1

The claimed priority date is not valid for the following reasons :

The following features in independent claim 1, resp. independent claim 11, represent an extension of subject matter with respect to the content of the priority document:

- claim 1: "acquisition of at least one image in a single color scale"; and "a separation of the plurality of particles using a color threshold"
- claim 13: "an image capture device for capturing a single color image ..."

In effect, the priority document mentions only the acquisition and thresholding of an electron microscope image, which is implicitly a gray-scale image (see in particular p. 3, lines 24 - p. 4, line 16; p. 5, line 31 - p. 6, line 4 of the priority document). The aforementioned features in claims 1 and 11 could however be interpreted in terms of the use of a color filter placed in front of the CCD camera for acquiring images in any particular wavelength band (color). Such extension of subject matter is not supported by the priority document.

The combination of features defined in independent claims 1 and 11 and, following, in

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP 03/08446

dependent claims 2-10 and 12-14 is therefore not unambiguously derivable from the priority document.

The effective priority date of the application is therefore the filing date, i.e. 30 July 2003, with effect that document D1, made available online on 7 Feb 2003 (otherwise paper-published on March 2003) constitutes prior art according to Art. 33(2) PCT.

2.2

Document D1, by applicant, discloses the subject matter of claims 1 and 11, the term "color scale", "color threshold", "color image" in these claims being interpreted - according to the description on page 6, lines 25-34 - as grey scale values (incidentally, claims 1 and 11 are not supported by the description for similar reasons as explained above with respect to the priority document. Claims 1 and 11 thus do not meet the requirements of Art. 6 PCT):

Claim 1 : see Abstract, D1.

Claim 11 : see Fig. 1, D1.

It follows that claims 1 and 11 do not meet the requirements of Art. 33(2) PCT in respect of novelty.

2.3

The additional features defined in dependent claims 2-10 and 12-14 are also known from D1 (Art. 33(2) PCT), see in particular Abstract, p. 230, col. 1, second paragraph - p. 231, col. 2, third paragraph and Figs. 1, 5, D1.

Re Item VIII

Certain observations on the international application

Beyond the reasons already mentioned in above paragraph 2.2, claims 1 and 11 also do not meet the requirements of Art. 6 PCT because, according to the description, the alleged invention is limited to a computerized method for identifying and classifying particles, said method further pertaining to the field of electron microscopy. These limitations are however not apparent from the claims. In particular, claim 1 as formulated would not exclude a method based on "manual" identification and classification. Also, both claims 1 and 11 could be interpreted according to document D2, which pertains to particle analysis using optical microscopy (indeed, D2 takes away the novelty of both claims 1 and 11 - Art. 33(2) PCT - see the corresponding passages

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP 03/08446

of D2 cited in the search report)

Claims 1 and 11 are thus not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description and drawings.

Claims

1. Method for multiple labeling detection and evaluation of a plurality of particles in specimen analysis comprising the steps of:
 - i) acquisition of at least one image in a single color scale;
 - ii) a separation of the plurality of particles using a color threshold;
 - iii) identification and classification of one or more particle types of the plurality of the particles to form different particle classes; and
 - iv) visualization of locations of the plurality of particles
- 5 2. Method according to claim 1, wherein the acquisition of contrast images is made by a slow scan cooled charge couple device camera.
- 10 3. Method of claim 2, wherein the images are at least 14 bit images.
- 15 4. Method of any one of the above claims, wherein the separation of the plurality of particles is carried out with reference to an underlying background image.
- 20 5. Method of any one of the above claims, wherein the separation of the plurality of particles is carried out with reference to specimen structures.
- 25 6. Method of any one of the above claims, wherein the identification and classification of the one or more particle types is dependent on shape of the particle types.
7. Method of any one of the above claims, wherein the identification and classification of the one or more particle types is dependent on size of the particle types.
- 30 8. Method of any one of the above claims, wherein the images are processed using at least 16 bits.

9. Method according to any of the above claims, wherein the image can be a photo-montage of a plurality of the images.
10. Method according to any one of the above claims, wherein the visualization of the location of the plurality of particle is carried out by a false coloring of the plurality of particles.
11. Method according to claim 10, wherein different false colors are used for different particle classes.
12. Method according to any one of the above claims, further comprising the generation of an overlay image.
13. Apparatus for the analysis of particles comprising:
 - an image capture device for capturing a single color image of a specimen with particles;
 - an image enhancement device;
 - an image identification and classification device; and
 - a display device for visualizing the specimen with the particles.
14. Apparatus according to claim 13, wherein the image capture device is a camera connected to an electron microscope.
15. Apparatus according to claim 14, wherein the camera is a slow scan charge coupled device.
16. Apparatus according to any one of claims 13 to 15, wherein the image identification and classification device classifies the particles on the basis of size of the particles.
17. Apparatus according to any one of claims 13 to 16, wherein the image identification and classification device classifies the particles on the basis of shape of the particles.

- REPLACED BY
ART 34 AMDT
18. Apparatus according to any one of claims 13 to 17, wherein the display devices visualise the particles in false colors.